Osteoporosis in Children and Young Adults

A Late Effect After Chemotherapy for Bone Sarcoma

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Abstract

Background Premature bone loss after childhood chemotherapy may be underestimated in patients with bone sarcoma. Methotrexate (MTX), a standard agent in osteosarcoma protocols, reportedly reduces bone mineral density (BMD). The literature, however, has reported cases of BMD reduction in patients with Ewing's sarcoma treated without MTX. Thus, it is unclear whether osteoporosis after chemotherapy relates to MTX or to other factors.

Questions/purposes We therefore asked whether (1) young patients with a bone sarcoma had BMD reduction, (2) patients treated with MTX had lower BMD, and

(3) other factors (eg, lactose intolerance or vitamin D deficiency) posed additional risks for low BMD.

Methods We retrospectively reviewed 43 patients with malignancies who had dual-energy x-ray absorptiometry (DEXA) (lumbar, femoral); 18 with Ewing's sarcoma (mean age, 26 ± 8 years), and 25 with an osteosarcoma (mean age, 27 ± 10 years). The mean time since diagnosis was 8 ± 4 years in the group with Ewing's sarcoma and 7 ± 5 years in the group with osteosarcoma. At last followup we determined BMD (computing z-scores), fracture rate, and lifestyle, and performed serum analysis.

Results BMD reduction was present in 58% of patients (37% had a z-score between -1 and -2 SD, 21% had a z-score less than -2 SD) in at least one measured site. Seven of the 43 patients (16%) had nontrauma or tumor-associated fractures after chemotherapy. Findings were similar in the Ewing and osteosarcoma subgroups. We found vitamin D deficiency in 38 patients (88%) and borderline elevated bone metabolism; lactose intolerance was present in 16 patients (37%).

Conclusion Doctors should be aware of the possibility of major bone loss after chemotherapy with a risk of pathologic fracture. Vitamin D deficiency, calcium malnutrition, and lactose intolerance may potentiate the negative effects of chemotherapy, and should be considered in long-term patient management.

Level of Evidence Level II, prognostic study. See Guidelines for Authors for a complete description of levels of evidence.

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Introduction

With an estimated incidence of four to five cases per million, osteosarcoma is the most common, nonhemopoietic primary malignant bone tumor, developing most frequently

in the second decade of life, with approximately 60% of patients being younger than 25 years [19]. Primitive neuroectodermal tumor (PNET) and Ewing's sarcoma are the second most common sarcomatous tumors of bone and soft tissue in young patients, with a peak age incidence in the second decade during maximal bone growth [19]. Both tumors affect the long bones of the appendicular skeleton and occur with occult metastases at the time of diagnosis in as much as 90% of cases [3]. Chemotherapy, following standardized study protocols [3, 4, 6, 7, 15, 21, 31], and potential radiotherapy (Ewing's sarcoma) in addition to wide resection have been seen to increase patients' survival [19]. Although the 5-year survival rate for patients with nonmetastasized Ewing's sarcoma has been less than 10% with resection only (with or without radiotherapy) [3], it has reached 50% to 75% with additional chemotherapeutic treatment [3]. A similar increase in survival has been reported for patients with osteosarcoma, for whom the 5-year survival increased from 15% to 50% to 70% [3].

Although chemotherapy is now an essential and powerful part of sarcoma treatment, it also has created a generation of young childhood sarcoma survivors who now are facing long-term effects of early cancer treatment. These patients reportedly have lower peak bone mass with consequently lower bone mineral density (BMD) later in life, followed by premature osteopenia and/or osteoporosis, and a higher risk of osteoporotic fractures [23, 34, 40, 41].

High-dose methotrexate (HD-MTX), a standard agent in the therapy protocols for osteosarcoma, has long been suspected as one of the main triggers of negative long-term effects on bone metabolism and BMD. MTX osteopathy was described in eight of 87 patients with osteosarcoma with at least three of four radiographic abnormalities including osteopenia, dense zones of provisional calcification, insufficiency fractures, and involvement of multiple bones [8]. Among 48 long-term survivors who had DEXA, 10 had osteoporosis, 21 had osteopenia, and 18 had fractures after chemotherapy [17]. However, Ruza et al. questioned MTX as one of the most suspect chemotherapeutics causing premature bone loss because the BMD of patients with Ewing's sarcoma treated without MTX was

reportedly decreased [33]. Thus, it is unclear whether MTX is one of the main triggers of bone loss after chemotherapy or if other factors also play an important role.

We therefore asked whether (1) young patients with bone sarcomas had BMD reduction, (2) patients treated with MTX had lower BMD, and (3) other factors (eg, lactose intolerance or vitamin D deficiency) posed additional risks for low BMD.

Patients and Methods

To evaluate BMD and laboratory alterations in young survivors of bone sarcomas and perform a two-group comparison of patients with Ewing's sarcoma and osteosarcoma, we queried our departmental database (established in 1998). We identified 103 patients treated between 1998 and 2009, and excluded 26 patients who were older than 50 years at the time of the study to minimize the possible influences of menopausal or senile BMD reduction, assumed to arise earliest at approximately this age [14]. This left 77 patients (38 with Ewing's sarcoma and 39 with osteosarcomas) who had completed chemotherapy and were younger than 50 years at the time of the investigation. Using either the telephone and/or letters, we invited these 77 patients to participate in our study, including densitometry, laboratory examination, and a lifestyle questionnaire. Fifteen patients were lost to followup (five from other countries) and 13 declined participation. These exclusions left 49 patients (64%) who agreed to participate: 43 at our institution and six at an outside hospital. We excluded the six patients from an outside hospital to eliminate technical differences in DEXA scans and laboratory evaluations. Therefore, the 43 patients with densitometry and laboratory studies at our institution represented the final study collective (Table 1), including 18 patients with Ewing's sarcoma (male-to-female ratio, 10:8) with a mean age of 26 ± 8 years (range, 12–44 years) and 25 patients with an osteosarcoma (male-to-female ratio, 16:9) with a mean age of 27 ± 10 years (range, 7–49 years). The tumors affected a solitary site and were located in the lower limbs in 34 patients, in the upper limbs in four, and in

Table 1. Patient characteristics

Characteristic	Ewing's sarcoma (n = 18)	Osteosarcoma (n = 25)	Total (n = 43)
Male: female	10:8	16:9	26:17
Mean age at diagnosis (range) (years)	18 ± 9 (3–38)	20 ± 11 (6–45)	19 ± 11 (3–45)
Mean age at followup (range) (years)	26 ± 8 (12–44)	27 ± 10 (7–49)	27 ± 9 (7–49)
Mean time between diagnoses and followup (years)	8 ± 4	7 ± 5	7 ± 5
Mean height (range) (cm)	169 ± 12 (130–191)	172 ± 14 (123–195)	171 ± 14 (123–195)
Mean weight (range) (kg)	61 ± 16 (25–95)	67 ± 19 (26–131)	64 ± 18 (25–131)
Mean BMI (range) (kg/m^2)	21 ± 4 (15–29)	22 ± 6 (16–45)	22 ± 5 (15–45)

other sites than the extremities in five; one patient with Ewing's sarcoma had multiple lesions. Metastases in the lungs were found in three patients with Ewing's sarcoma and three with osteosarcoma at the time of diagnosis. The mean followup was 8 ± 4 years (range, 3–18 years) in the group with Ewing's sarcoma and 7 ± 5 years (range, 1–17 years) in the group with osteosarcoma. The local ethics committee

approved the study protocol, and all patients gave written informed consent to participate in the study.

All 43 patients had received chemotherapy according to the treatment protocols (Fig. 1) active at the time of diagnosis, in addition to a wide resection, except one patient with Ewing's sarcoma and one with osteosarcoma who had inoperable tumors. Eight patients, six with

Chemotherapeutic Protocols - Ewing´s Sarcoma				
CESS (till 1992) [7]	VACA		VAIA	
	Vincristine Actinomycin-D Cyclophosphamide Ifosfamide Adriamycin (=Doxorubicin)			
	CESS ± Etoposide			
Euro-Ewing 99 [3]	Neoadjuvant	Adjuvant		
	VIDE	VAI	VAC	Bu-Mel
	Vincristine	Vincristine		Busulfan
	Ifosfamide	Actinomycin-D		Melphalan
	Doxorubicin	Ifosfamide	Cyclophosphamide	
	Etoposide			
A				
Chemotherapeutic Protocols – Osteosarcoma				
COSS (till 2005) [3]	Doxorubicin Cisplatin HD-MTX (+Leucovorin Rescue) Ifosfamide			
EURAMOS 1 (below 40 years) [3]	COSS + Interferon α			
EURO-B.O.S.S. (over 40 years) [3]	Neoadjuvant	Adjuvant		
		Good / Intermediate Responders		Bad Responders
	Doxorubicin	Doxorubicin		Doxorubicin
	Cisplatin	Cisplatin		Cisplatin
	Ifosfamide	Ifosfamide		Ifosfamide HD-MTX
B				

Fig. 1A–B The chemotherapeutic treatment guidelines for Ewing's sarcoma and osteosarcoma according to study proven protocols are shown. (**A**) The graph shows the chemotherapeutic protocols and

major chemotherapeutic agents used for treatment of Ewing's sarcoma [3, 7, 15, 31] and (**B**) osteosarcoma [3, 6, 15, 21].

Ewing's sarcoma and two with osteosarcomas, also received local radiotherapy: two because of inoperability and six because of anatomically problematic wide resection and partially bad response to chemotherapy.

To gain information regarding patients' lifestyles, family history, other bone-affecting diseases, and intake of osteoreductive medication, we interviewed them according to a specially adapted questionnaire originally described by Obermayer-Pietsch et al. [27] (Table 2).

DEXA currently is the most commonly used technique to quantify BMD in clinical practice, and therefore was our method of choice [9]. We based our evaluation on the z-score system to compare BMD values of our young patients in conjunction with the t-score, used for the WHO osteoporosis definition [34, 41]. The main difference between the two scores was the control group to which the DEXA result was related [41]. In general, we believed premature bone loss in young patients was better described as low bone density for chronologic age than with the terms osteopenia and osteoporosis [2]. Nevertheless, for reasons of simplicity and comparability, we used z-scores between -1 SD and -2 SD as osteopenia and z-scores less than -2 SD as osteoporosis [34]. None of the eight patients who received radiotherapy had DEXA measurement at the site of radiation. The DEXA scans were obtained a minimum of 1 year after diagnosis (mean, 7 ± 5 years; range, 1–18 years).

Blood samples were taken in the morning after an overnight fast. The laboratory analyses included a general serum profile, bone metabolism, genetic analysis of lactose intolerance, and functional status of the thyroid gland, hypophysis, and gonads.

For calculations, we used R 2.12.0 (R foundation, Vienna, Austria). We assessed our patients' BMD by using the scoring system and compared laboratory results with standardized ranges. We determined differences in BMD and laboratory values between the Ewing's and osteosarcoma groups using the exact Wilcoxon rank sum test. To achieve more power compared with Bonferroni corrections, we used a global test according to Goeman et al. [12], which was developed for comparison of two groups with respect to many measurements. For evaluation of differences in lactose intolerance, we double checked using the exact Wilcoxon rank sum test and the model free likelihood-ratio test. To identify possible influencing factors on our patients' BMD, we tested correlations with Spearman's rank correlation coefficient (except for sex for which we used the Wilcoxon test), Bonferroni-corrected when necessary owing to multiple DEXA testing.

Results

Twenty-one percent of our patients had osteoporosis (z-score lower than -2 SD) and 37% had osteopenia (z-score

between -1 SD and -2 SD) in a least one measuring site (Fig. 2). We recorded the mean BMD values and z-scores (Table 3). In tumors affecting the weightbearing bones, we found a lower BMD in the limb with the primary tumor location (femoral neck, $p < 0.001$; total femur, $p = 0.0019$). We therefore concentrated our femoral evaluation on the unaffected side, but excluded six patients who had data available only for the affected limb. Fifteen of the 43 patients had 17 nonvertebral fractures, 14 attributable to trauma and three associated with tumors before chemotherapy (one patient in each tumor group had two fractures at different times and sites). After chemotherapy, seven of the 43 patients had nontraumatic and nontumor-associated fractures (Table 4). Five of these patients had low BMD levels in at least one site. We found normal baseline serum calcium values, whereas serum phosphate decreased below the reference limit in 10 patients and median levels of all 43 patients ranged in the lower third of the reference range (Table 5). Our patients showed increases in bone-specific alkaline phosphatase (86%), N-terminal telopeptide procollagen (60%), osteocalcin (47%), beta-CrossLaps (65%), tartrate resistant alkaline phosphatase (23%), and osteoprotegerin (26%), all with a general upward tendency, and decreased receptor activator of NF- κ B ligand (RANKL) levels (51%). Thyroid hormones were normal. Increases in growth hormone were observed in 10 patients and insulinlike growth factor-1 in 12 patients. Free (bioactive) testosterone in patients older than 17 years tended toward the lower reference limit paralleled by increased follicle stimulating hormone in 15 patients and luteinizing hormone in eight. Other than BMD reduction and laboratory disturbances, we found no common characteristics of patients with osteoporosis compared with those without.

Differences between the two tumor types trended ($p = 0.053$) toward lower BMD values in Ewing's sarcoma, especially in the lumbar spine. Creatinine ($p = 0.04$) and albumin ($p = 0.04$) were lower in patients with osteosarcoma.

We found vitamin D deficiency in 38 of the 43 (88%) patients with mean parathyroid hormone levels in the normal range. Genetically defined lactose intolerance (Table 6) occurred in 16 patients and was observed in similar proportions of patients with Ewing's sarcoma and osteosarcoma ($p = 0.363$) and did not correlate ($p = 1.00$) with the BMD measured. Greater height ($p = 0.03$), weight ($p < 0.001$), and BMI ($p < 0.001$) correlated with a greater BMD in all sites. Sex ($p = 0.29$), age at diagnosis ($p = 0.52$), and the time between chemotherapy and our investigation ($p = 0.21$) did not affect the outcome. In females, neither age at menarche ($p = 0.39$) nor the time between menarche and chemotherapy ($p = 1.00$) were influencing.

Table 2. Patient lifestyle and risk factors (questionnaire)

Family history	Physical demand (whole study group)	
0% increased incidence of fractures	Occupation	Sports
	72% sedentary 9% standing 19% in movement	21% no sports 32% sparse 42% regularly 5% > 1 hour per day
30% increased incidence of osteoporosis	Physical demand (in the 30% of patients with osteoporosis in family history)	
	Occupation	Sports
	70% sedentary 15% standing 15% in movement	31% no sports 31% sparse 38% regularly 0% > 1 hour per day
Smoking	Alcohol consumption	Sun exposure
3 active smokers (0.5-7.5 pack years) 1 former smoker (1 pack year)	32% no alcohol 63% occasional 5% regularly	65% rarely 33% regularly 2% often 0% solarium
Milk consumption	Cheese/dairy product consumption	Lactose intolerance
28% no milk 53% ≤ 0.25l per day 19% > 0.25l per day	40% occasional 60% daily	2% verified by H2-respiratory test 9% disturbed lactose digestion (subjective) 89% no problems with lactose digestion
Bone influencing medication and diseases		
Patients with Ewing's sarcoma	Patients with osteosarcoma	Patients with Ewing's and osteosarcoma
1 current Renitec® / Reducto spezial® treatment (toxic tubulopathy) 1 calcium and vitamin D (for 3 years) + bisphosphonates iv (2 times)	1 current Trileptal® / Seroquel® treatment (epilepsy) 1 corticosteroid (in course of CTX)	39 with no other diseases with an indirect osteoreductive influence 39 no other possibly bone harming medication
Female cycle (n = 17)	Ewing's sarcoma (n = 8)	Osteosarcoma (n = 9)
Mean age at menarche (range) (years)	12 years (10-14)	14 years (12-16)
Amenorrhea		
During CTX	63%	44%
At the time of investigation	37%	44%
Cycle at the time of investigation		
Regular	37%	44%
Irregular	25%	11%
Contraception at the time of investigation		
Estrogen-based	37%	11%
Progesteron-based	0%	11%
Pregnancies = births (no miscarriages)	3 (n = 2)	8 (n = 3)
Mean breast feeding time (months)	3.75	15

CTX = chemotherapy; Renitec® (Merck Sharp&Dohme GmbH, Vienna, Austria); Reducto spezial® (Temmler Pharma GmbH&Co.KG, Marburg, Germany); Trileptal® (Novartis Pharma GmbH, Vienna, Austria); Seroquel® (AstraZeneca GmbH, Vienna, Austria).

Fig. 2A–F The graphs show the results of DEXA measurement in reference to the z-score system. The percentage distributions of osteoporosis (dark), osteopenia (middle), and normal BMD (light) in (A) the lumbar spine, (B) the femoral neck, and (C) the total femur

Discussion

Bone loss after childhood chemotherapy might be underestimated in patients with bone sarcoma. HD-MTX, a standard agent in osteosarcoma protocols, reportedly has reduced BMD [8, 17]. The literature, however, shows decreased BMD in patients with Ewing's sarcoma treated without HD-MTX [33]. Thus, it is unclear whether

are presented. In context with patients' ages, the exact evaluated z-scores and their distribution among the two tumor entities (Ewing's sarcoma - green; osteosarcoma - blue) in the (D) lumbar spine, (E) the femoral neck, and (F) the total femur are shown.

osteoporosis after chemotherapy relates to HD-MTX or to other factors. We therefore evaluated whether (1) young patients with bone sarcoma had premature BMD reduction, (2) patients treated with MTX had lower BMD, and (3) other factors (eg, lactose intolerance or vitamin D deficiency) posed additional risks for low BMD.

Our study had several limitations. First, we had a relatively small number of participants owing to the rarity of

Table 3. Mean bone mineral densities (BMD) [g/cm²]

Parameter	Number	BMD (mean ± SD)	z-score (mean ± SD)
Lumbar spine L1–L4 (n = 43)			
Ewing's sarcoma (n = 18)			
Normal BMD	8	1.136 ± 0.135	−0.013 ± 0.591
Osteopenia	5	0.991 ± 0.036	−1.610 ± 0.123
Osteoporosis	5	0.880 ± 0.049	−2.455 ± 0.272
Osteosarcoma (n = 25)			
Normal BMD	17	1.173 ± 0.187	0.253 ± 0.869
Osteopenia	6	0.987 ± 0.052	−1.600 ± 0.302
Osteoporosis	2	0.981 ± 0.050	−2.238 ± 0.137
Total (n = 43)			
Normal BMD	25	1.161 ± 0.173	0.168 ± 0.801
Osteopenia	11	0.989 ± 0.045	−1.605 ± 0.238
Osteoporosis	7	0.909 ± 0.067	−2.393 ± 0.260
Femoral neck (tumor unaffected side) (n = 37)			
Ewing's sarcoma (n = 17)			
Normal BMD	8	0.971 ± 0.108	−0.075 ± 0.642
Osteopenia	6	0.851 ± 0.072	−1.483 ± 0.241
Osteoporosis	3	0.742 ± 0.021	−2.467 ± 0.386
Osteosarcoma (n = 20)			
Normal BMD	13	1.030 ± 0.160	0.192 ± 0.876
Osteopenia	6	0.831 ± 0.055	−1.476 ± 0.262
Osteoporosis	1	0.670 ± 0.000	−2.300 ± 0.000
Total (n = 37)			
Normal BMD	21	1.008 ± 0.146	0.090 ± 0.805
Osteopenia	12	0.841 ± 0.065	−1.475 ± 0.252
Osteoporosis	4	0.724 ± 0.036	−2.425 ± 0.342
Total femur (tumor unaffected side) (n = 37)			
Ewing's sarcoma (n = 17)			
Normal BMD	10	1.002 ± 0.111	−0.190 ± 0.536
Osteopenia	4	0.872 ± 0.045	−1.325 ± 0.228
Osteoporosis	3	0.717 ± 0.028	−2.567 ± 0.262
Osteosarcoma (n = 20)			
Normal BMD	13	1.044 ± 0.168	0.146 ± 0.918
Osteopenia	6	0.840 ± 0.055	−1.533 ± 0.221
Osteoporosis	1	0.638 ± 0.000	−2.700 ± 0.000
Total (n = 37)			
Normal BMD	23	1.026 ± 0.148	0.000 ± 0.793
Osteopenia	10	0.853 ± 0.054	−1.450 ± 0.246
Osteoporosis	4	0.698 ± 0.042	−2.600 ± 0.235

BMD = bone mineral density.

the tumor entities, mortality rate, and inclusion criteria concerning age. Second, the different intervals between chemotherapy and investigation varied from 9 months to 18 years. Nevertheless, this enabled us to further evaluate a possible correlation between time of remission and BMD. Third, six of the 43 patients were excluded from the femoral BMD analysis owing to the absence of bilateral femoral DEXA data. However, we still had a study

population of 43 patients with lumbar spine and laboratory data. Further, the lumbar spine has been assumed to be even more sensitive to therapeutic modalities owing to the large mass of trabecular bone [1, 11]. Fourth, we compared our DEXA data with a scoring system instead of using a normal study population, mainly for higher reliability because of the large number of patients included in the database. Fifth, we tested potential influencing factors on

Table 4. BMDs [g/cm²] and z-scores of patients with fractures

Location of fracture	Time after CTX (months)	Type of tumor	L1–L4		Femoral neck (tumor unaffected)		Total femur (tumor unaffected)	
			BMD	z-score	BMD	z-score	BMD	z-score
Proximal femur	36	OS	0.974	−1.2	0.670	−2.3	0.638	−2.7
Proximal femur	72	ES	0.842	−2.3	0.619*	−2.7	0.582*	−3.1
Distal femur	192	OS	1.065	−1.2	0.821*	−1.8	0.689*	−2.9
Distal femur, proximal tibia	29	ES	0.891	−2.7	0.759	−2.1	1.111	0.4
Proximal tibia	32	OS	1.102	−0.5	0.962	−0.3	0.989	−0.3
Proximal tibia	30	OS	0.921	0.1	0.563*	−3.2	0.548*	−3.1
Finger	72	OS	1.415	1.6	1.102	0.9	1.133	1.0

* Data for the tumor-affected side; CTX = chemotherapy; BMD = bone mineral density; ES = Ewing's sarcoma; OS = osteosarcoma.

BMD only in univariable analysis, finding height, weight, and BMI influential. In a multivariate analysis, some of those factors may not independently predict BMD. Sixth, our study was set in spring, when seasonal vitamin D deficiency may have overshadowed the influence of chemotherapy. Nevertheless, we diagnosed a high percentage of patients with BMD reduction, which was unlikely to be attributable to seasonal vitamin D imbalances.

We found decreased bone mineral density levels in 25 of the 43 patients (58%) compared with age- and gender-related pairs in at least one measuring point. We also compared our data with that in the literature (Table 7). The studies varied in study population and followup times; nevertheless, both revealed a remarkable number of patients with BMD reduction and fractures [17, 33], which are often the first indicator of bone loss. The estimated lifetime-fracture risk attributable to osteoporosis reportedly is exponentially related to BMD, which decreases up to 10-fold faster if related to tumor treatment, especially in association with hypogonadism [13]. One study suggested an association between osteoporotic fractures in patients' history and a higher risk for subsequent events [13]. However, in children, the absence of fractures did not automatically imply an age-related healthy bone status because they often did not fracture, even if very low z-score levels were reached [22]. Refinement of our DEXA scans was reached by laboratory evaluation. Our patients had an increase in bone-specific serum parameters, indicating higher rates of skeletal remodeling. This coincided with findings in the literature, where lower rates of skeletal remodeling correlated with reaching higher bone mass during puberty [37]. A negative correlation between markers of bone formation and resorption to BMD also was reported in one study [33], whereas in another study this correlation was seen only concerning bone resorption [17]. Increased growth hormone and insulinlike growth factor-1, both important for bone growth (childhood) and maintenance (adulthood), further correlated with higher

bone metabolism [10, 32, 38], documenting the growth period of some of our patients.

Consistent with one report [33], our observations did not support the notion that patients with osteosarcoma who had HD-MTX in their treatment schemes have lower BMD levels than patients with Ewing's sarcoma treated without HD-MTX. Rather, we observed a trend in Ewing's sarcoma toward lower BMD levels, especially in the lumbar spine, which seemed to present a higher sensitivity to osteopenic agents because of the trabecular bone as the main component [1, 11]. Longer average treatment duration in Ewing's sarcoma (10–12 months) compared with osteosarcoma (6–12 months) might have contributed to this outcome as well [15].

We found a high percentage of vitamin D deficiency (88%) which might have contributed to the observed increase in bone-turnover. Inadequate vitamin D levels have been related to children's inability of attaining their genetically programmed peak bone mass, and, in adults, vitamin D deficiency has led to severe bone disturbances [16, 29, 39]. Secondary hyperparathyroidism attributable to vitamin D deficiency with consecutive renal loss of phosphate may have resulted in an increased bone-turnover, impaired mineralization, and loss of BMD, along with higher incidences of fractures [24, 28, 30]. This may explain why bone loss was unaffected by sex, age at the time of chemotherapy, and followup time. Additionally, we found a relatively high percentage of patients with genetic determination for lactose intolerance (37%) as compared with the Central European average (20%–40%) [27]. Patients' tending to avoid intolerance symptoms by reducing calcium-rich dairy products might have had a greater impact than the intolerance [5, 18, 20, 25]. Low calcium intake would correlate with an increase in serum parathyroid hormone and increased bone turnover [35]. Serum calcium measurements were not suitable to prove sufficient nutritional calcium supply because changes may have occurred

Table 5. Laboratory values

General laboratory values	Ewing's sarcoma (n = 18)	Osteosarcoma (n = 25)	Total (n = 43)	p value
Electrolytes				
Total calcium (2.2–2.65 mmol/L)	2.4 (2.3–2.6)	2.4 (2.2–2.6)	2.4 (2.2–2.6)	0.46
Phosphate (2.6–4.5 mg/dL)	2.7 (1.9–4.1)	3.3 (2.2–4.4)	3.1 (1.9–4.4)	0.10
Kidney				
Creatinine (0.5–1.0 mg/dL)	1.1 (0.8–1.8)	0.9 (0.4–1.3)	1 (0.4–1.8)	0.04
Urea (10–45 mg/dL)	30.5 (16–57)	30 (19–55)	30 (16–57)	0.72
Blood urea nitrogen (mg/dL)	14.2 (7.5–26.6)	14 (8.9–25.7)	14 (7.5–26.6)	0.72
Liver				
Gamma-glutamyl transferase (–38 U/L)	15.5 (10–511)	17 (7–115)	17 (7–511)	0.58
Aspartate aminotransferase (–30 U/L)	29 (17–93)	25 (17–56)	27 (17–93)	0.49
Alanine aminotransferase (–35 U/L)	21.5 (11–186)	22 (10–119)	22 (10–186)	0.90
Serum proteins				
Total globulin (6.6–8.3 g/dL)	7.9 (6.5–9.1)	7.7 (6.8–9.0)	7.7 (6.5–9.1)	0.34
Albumin (3.5–5.3 g/dL)	5.2 (4.2–5.9)	5.0 (4.2–5.5)	5.1 (4.2–5.9)	0.04
Endocrinology				
Thyroid gland				
Thyroid stimulating hormone (0.1–4 µU/mL)	1.5 (1.1–3.3)	1.6 (0.4–2.7)	1.6 (0.4–3.3)	0.53
Triiodothyronine (3–6.3 pmol/L)	5.3 (4.1–6.4)	5.2 (4–6.8)	5.2 (4–6.8)	0.25
Thyroxine (9.5–24 pmol/L)	15.9 (12.1–22.9)	14.4 (11.2–24.2)	14.8 (11.2–24.2)	0.98
Parathyroid gland/bone metabolism				
Parathyroid hormone (15–65 pg/mL)	38.1 (18.7–65.6)	45.7 (21.8–77.8)	43.3 (18.7–77.8)	0.45
25 (OH) vitamin D3 (30–60 ng/mL)	15.8 (5–50.2)	16.6 (7.6–104.6)	16.4 (5–104.6)	0.47
Bone-specific alkaline phosphatase (µg/L)*	31.4 (16.6–75.5)	29.4 (15.8–97.8)	31.1 (15.8–97.8)	0.58
Osteocalcin (1–35 ng/mL)	30.7 (1.5–255.7)	34.4 (15–168.2)	31.9 (1.5–255.7)	0.79
N-terminal telopeptide procollagen (ng/mL)†	62.4 (29.7–417.3)	81.1 (31.5–722.4)	78.8 (29.7–722.4)	0.52
Osteoprotegerin (0.7–4.2 pmol/L)	3.2 (0.1–7.0)	3.6 (1.3–7.7)	3.5 (0.1–7.7)	0.33
RANKL (0.04–0.85 pmol/L)	0.04 (0.0–0.4)	0.02 (0.0–1.1)	0.03 (0–1.1)	0.56
Tartrate resistant alkaline phosphatase (U/L)‡	3.1 (1.7–5.6)	3.4 (2.4–11.5)	3.3 (1.7–11.5)	0.21
Beta-CrossLaps (ng/mL)§	0.7 (0.2–3.7)	0.6 (0.3–2.8)	0.6 (0.2–3.7)	0.69
Gonads				
Testosterone total (♂) (2.41–8.3 ng/mL)	4.7 (0.2–8.8)	4.8 (0.1–8.8)	4.7 (0.1–8.8)	0.70
Testosterone free (♂) (6.69–54.69 pg/mL)	18.2 (2.4–34.3)	15.7 (0.2–28.1)	16.4 (0.2–34.3)	0.39
Sexual hormone binding protein (♂) (16–76 nmol/L)	37.1 (22.3–111.1)	37 (23.3–200)	37.1 (22.3–200)	0.78
Testosterone total (♀) (ng/mL)	0.3 (0.2–0.6)	0.4 (0.1–0.6)	0.4 (0.1–0.6)	0.76
Testosterone free (♀) (pg/mL)	1.5 (1–2.9)	1.7 (0.8–3)	1.6 (0.8–3)	0.89
Sexual hormone binding globulin (♀) (24–230 nmol/L)*†	118.6 (35.1–173.8)	47.1 (22.8–133.6)	88.5 (22.8–173.8)	0.07
Neuroendocrinology				
Growth hormone (0–7 ng/mL)	2.8 (0.2–11.8)	0.6 (0.1–15.7)	1.3 (0.1–15.7)	0.33
Insulinlike growth factor-1 (100–300 ng/mL)	230 (112–489)	242 (72.6–418)	238 (72.6–489)	0.59
Gynecologic/gonadal laboratory				
Follicle stimulating hormone (♂) (1.3–12 mIU/mL)	6.3 (1–31.9)	8.9 (0.9–33.7)	6.8 (0.9–33.7)	0.68
Follicle stimulating hormone (♀) (< 0.1–67.5 mIU/mL)	40.4 (0.3–115.4)	7.3 (2.4–183.7)	13.3 (0.3–183.7)	0.28
Luteotrope hormone (♂) (0.8–8.5 mIU/mL)	6.3 (0.4–13.8)	4.5 (0.3–16)	5.6 (0.3–16)	0.20
Luteotrope hormone (♀) (1.1–20 mIU/mL)	18.2 (0.3–48.4)	5.6 (1.7–71.8)	13.8 (0.3–71.8)	0.82

* 7.5–20.6 µg/L (♂), 5.8–14.8 µg/L (♀); † 16–67 ng/mL (♂), 20–75 ng/mL (♀); ‡ 2.18–3.94 U/L (♂), 1.81–3.37 U/L (♀); § 0.08–0.46 ng/mL (♂), 0.03–0.37 ng/mL (♀); * 89–379 nmol/L with oral contraceptives; all female ranges are valid for premenopausal women.

Table 6. Relation of lactose intolerance and measured BMD*

Lactose intolerance	Number of incidences (n = 43)	Lumbar spine	Number of incidences (n = 37)	Femoral neck	Total femur
TT	10	1.086 ± 0.183	9	0.948 ± 0.188	0.952 ± 0.209
TC	17	1.052 ± 0.092	14	0.919 ± 0.112	0.934 ± 0.125
CC	16	1.096 ± 0.220	14	0.910 ± 0.166	0.948 ± 0.169

BMD = bone mineral density; * mean BMD values ± SD; TT = no genetic determination for lactose intolerance; TC = little to no genetic determination for lactose intolerance; CC = genetic determination for lactose intolerance.

Table 7. Comparison of our study data with the literature

Study	Study participants					Mean age at followup (years) (± SD)		Mean followup times (years) (± SD)		Tumor localization in weightbearing bones (%)	
	Total	Ewing's sarcoma	Osteosarcoma	Male	Female	Ewing's sarcoma	Osteosarcoma	Ewing's sarcoma	Osteosarcoma	Yes	No
Holzer et al. [17]	48	0	48	22	26	NA	31 (± 4.24)	NA	16 (± 2.2)	92%	8%
Ruza et al. [33]	63	25	38	32	31	19.13 (± 4.20)	20.65 (± 4.42)	6 (± 3.73)	6 (± 3.67)	83%	17%
Current study	43	18	25	26	17	26.09 (± 7.61)	27.21 (± 10.34)	8 (± 4.2)	7 (± 4.7)	79%	21%
	Fractures		Number of patients (%)			Score	Method	Measuring sites			
	Before CTX	After CTX	Normal BMD	Osteopenia	Osteoporosis						
Holzer et al. [17]	16	18	17 (35%)	21 (43.7%)	10 (20.8%)	t-score	DEXA	lumbar spine, proximal femur (nonoperated side)			
Ruza et al. [33]	17 total		36 (57.1%)*	21 (33.3%)*	6 (9.52%)*	z-score	DEXA	lumbar spine (*), femoral neck (tumor unaffected side) (°)			
			36 (57.1%)°	16 (25.4%)°	11 (17.5%)°						
Current study	17	7	18 (41.9%)	16 (37.2%)	9 (20.9%)	z-score	DEXA	lumbar spine; femoral neck, total femur (tumor unaffected side)			

CTX = chemotherapy; BMD = bone mineral density; NA = not available.

by increased bone mobilization attributable to immobilization. Physical inactivity possibly influenced femoral BMD reduction in both groups, and was supposed to show higher impact on weightbearing bones than on the lumbar spine [1, 26]. Bone loss attributable to immobilization has been reported to be greatest during the first weeks of disuse, and its duration strongly affected the amount of BMD reduction [36]. In this context, a patient's attitude to favor the surgically treated limb [17] provided a reasonable explanation for the lower BMD levels measured in the tumor-affected leg compared with the unaffected one.

Our data support the notions that (1) premature reduction of BMD in young survivors of bone sarcoma should be

considered in a patient's management, (2) BMD deficits occurred independently from the tumor entity, and (3) BMD reduction possibly was potentiated by vitamin D deficiency and lactose intolerance. Therefore, we suggest regular endocrinology consultations and eventual adequate osteoprotective treatment; we recommend vitamin D and calcium. Owing to the results of our investigation, we offered consultation and treatment to the patients in this study and for all future patients.

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